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by

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ABSTRACT

Effective UV attenuation in the outer leaf layers may represent an important protective mechanism against potentially damaging solar UV-B radiation. Epidermal optical properties for Rumex patientia and Rumex obtusifolius were examined on field collected and greenhouse grown plants. Rumex patientia, a relatively UV-B sensitive plant, has substantially higher epidermal UV transmittance than Rumex obtusifolius, which indicated that the UV-B flux at the mesophyll layer for Rumex patientia would also be higher. Attenuation of UV-B radiation increased in Rumex obtusifolius by 27% after exposure to solar UV-B radiation. Flavonoid extract absorbance also increased in whole leaves of both species after solar UV-B irradiation. The epidermis is not only an effective filter for UV-B radiation, but is wavelength selective, and shows a degree of plasticity in this attenuation.

INTRODUCTION

Leaves are among the primary organs of higher plants for the interception of visible radiation. Morphological characteristics such as leaf arrangement on the stem, orientation, and structure are adaptations that often maximize the interception of light and its penetration to the mesophyll for photosynthesis. These same characteristics may not always afford effective protection from potentially injurious solar ultraviolet-B radiation (290-320 nm). Leaf optical properties that allow high transmittance of visible radiation, while selectively filtering out UV-B radiation before it penetrates to sensitive physiological targets in the leaf, present a viable protective mechanism for reducing UV-B injury.

Plant sensitivity to UV-B-induced damage varies considerably among species (Biggs et al. 1975; Van and Garrard 1975; Teramura 1983). These differences may, in part, be related to the effectiveness of factors in leaf tissue that affect UV-B attenuation. Several studies have shown that leaf surface reflectance of UV-B radiation and the capacity for UV-B attenuation in the outer leaf layers differs considerably among species (Lautenschlager-Fleury 1955; Gausman et al. 1975; Robberecht and Caldwell 1978; Robberecht et al. 1980). A mechanism that combines attributes such as effective UV-B attenuation, wavelength selectivity, and a degree of plasticity to respond to different UV-B irradiances would be of significant adaptive value. The role of UV-absorbing pigments such as flavonoids and

related phenolic compounds as a mechanism for plant acclimation to solar UV-B radiation has been suggested (Caldwell 1968; McCree and Keener 1974; Gausman et al. 1975; Wellmann 1975; Lee and Lowry 1980; Robberecht and Caldwell 1983). Plasticity in such a mechanism has significance not only for species that colonize high UV-B irradiance habitats, but also for plant growth in the intensified UV-B irradiance environment expected as a consequence of reduced stratospheric ozone (National Academy of Sciences 1982). The present study examined the leaf optical properties of two Rumex species in regard to a possible protective mechanism against damage induced by UV-B radiation. Rumex patientia L. has been shown to be a relatively UV-B-sensitive species (Sisson and Caldwell 1976), whereas R. obtusifolius L. appears relatively less sensitive (Sisson unpublished data). The two species differ significantly in epidermal UV optical properties for both field and greenhouse-grown plants.

MATERIALS AND METHODS

Epidermal UV transmittance was determined for fresh tissue obtained from greenhouse-grown plants and from plants collected in foothill habitats of northern Utah. The epidermis was mechanically removed. Transmittance and reflectance were measured with the integrating sphere/spectroradiometer system described in detail by Robberecht and Caldwell (1978). Absorptance was calculated by subtracting the sum of transmittance and reflectance from 100%.

A methanol-water-HCl solution (70:29:1 v/v) was used to examine the contribution of UV-absorbing pigments, such as flavonoids and related phenolic compounds, to the UV attenuating capability of the epidermis. This method effectively removes such compounds from leaf tissue (see Caldwell 1968; Ribereau-Gayon 1972). Fresh epidermal tissue was immersed in this solution for 15 minutes and subsequently re-examined for transmittance. Whole-leaf extracts were obtained for analysis of possible changes in flavonoid absorbance after exposure to solar UV-B radiation. Leaf tissue was ground in 5 ml of the extraction solution and centrifuged for 10 minutes. The resulting supernatant was then examined spectrophotometrically (Beckman Model 35) for absorbance.

Greenhouse cultivated plants of both species were transferred to the field and placed beneath either a 0.08-mm Aclar or 0.13-mm Mylar plastic film (DuPont Co.). Mylar excludes UV-B radiation, whereas Aclar is relatively transparent in this waveband (Fig. 1). The species were examined successively. Ultraviolet irradiance was weighted with the generalized plant spectrum normalized at 280 nm (Caldwell 1971). Mean biologically effective UV-B irradiation beneath the Aclar filter was 1125 J m^{-2} d^{-1} for Rumex patientia and 1151 J m^{-2} d^{-1} for R. obtusifolius. Determination of epidermal transmittance and whole-leaf extract absorbance were made at the end of the UV-B exposure period.

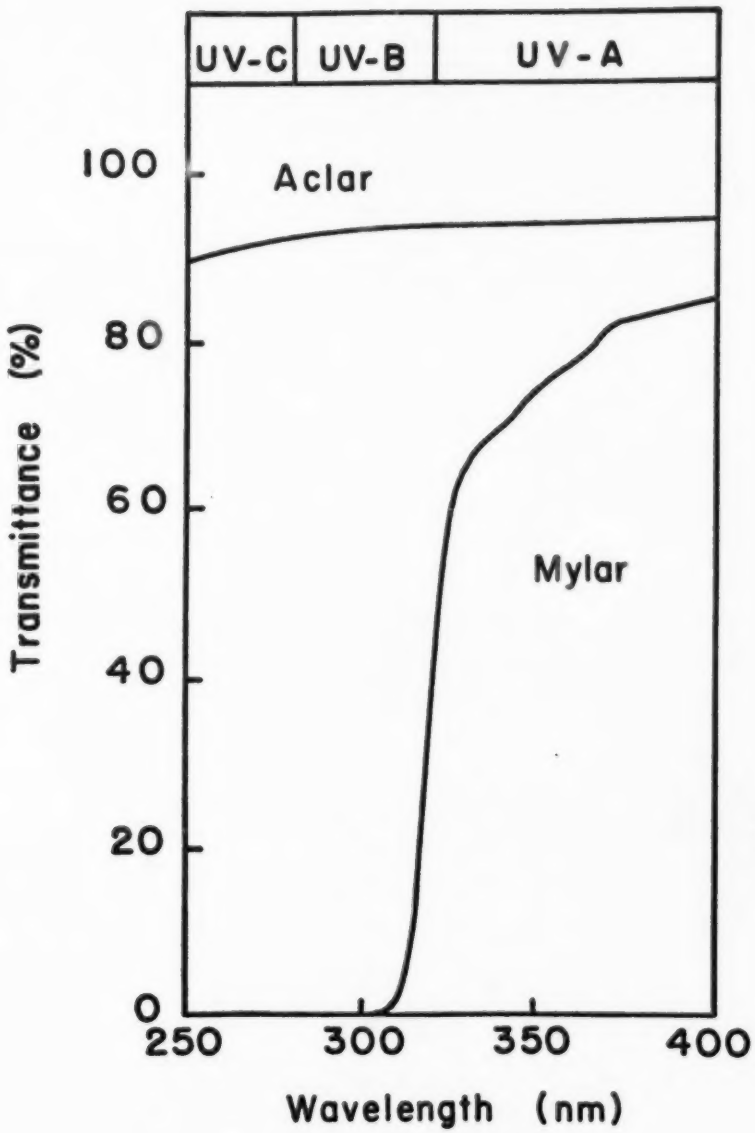

Fig. 1. Transmittance spectra of Aclar (0.08 mm) and Mylar (0.13 mm) plastic films.

RESULTS

Optical Properties

The two Rumex species differed most significantly in the degree of UV-B radiation transmitted through the epidermis to the mesophyll (Fig. 2). Mean epidermal UV-B transmittance for greenhouse-grown plants was 18% for R. patientia and 10% for R. obtusifolius. Ultraviolet-B reflectance of 4% was similarly low for both species, and showed little variation with wavelength in the UV waveband. The epidermis absorbed more than 80% of the incident UV-B radiation on the leaf.

Examination of field-grown plants also showed epidermal transmittance for Rumex patientia to be substantially greater than that for R. obtusifolius. After UV-absorbing compounds had been extracted from the tissue, UV-B transmittance was similar for both species (Fig. 3). Removal of UV-B-absorbing compounds increased mean UV-B transmittance from 8% for fresh tissue to 51% after extraction for R. obtusifolius, and similarly from 24% to 52% for R. patientia.

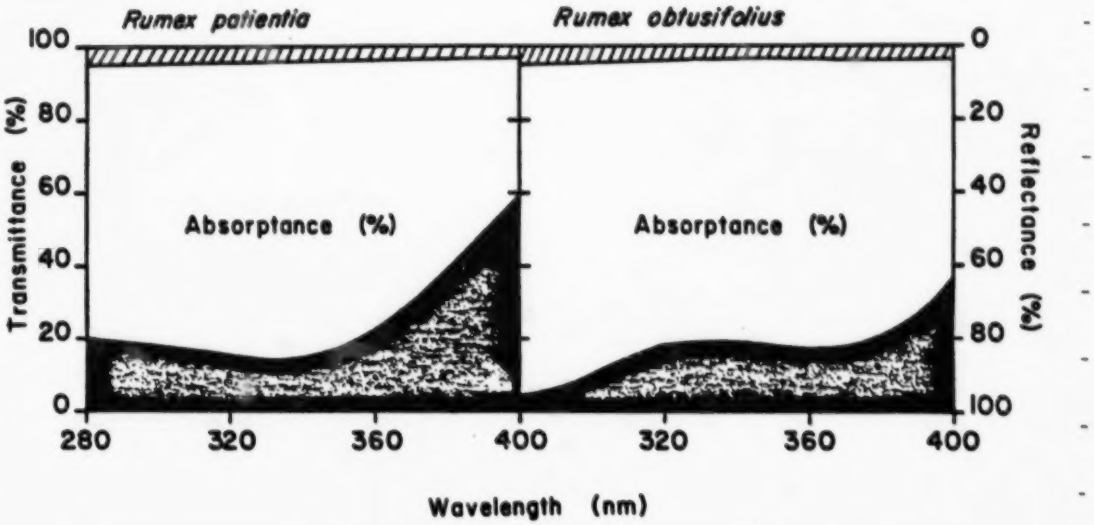

Fig. 2. Epidermal UV optical properties of two Rumex species cultivated under greenhouse conditions. Transmittance is indicated by the solid area and reflectance by the hatched area. Each spectrum represents the mean of three samples.

Influence of Solar UV-B Radiation

Epidermal UV-B transmittance for Rumex obtusifolius was significantly reduced ($p \leq 0.05$) by 27% after eight days of exposure to solar UV-B radiation (Fig. 4). This occurred in fully expanded leaves on plants that were transferred from the greenhouse to Aclar-covered frames in the field. After five days of solar UV-B irradiation, the epidermis of R. patientia

became brittle and prone to disintegration upon removal. Since epidermal samples of sufficient size for examination could not be obtained, no transmittance measurements were possible for this species.

Ultraviolet-B absorbance in extracts from whole-leaf tissue showed significant increases ($p \leq 0.05$) in response to solar UV-B exposure (Table 1). Absorbance increased an average of 11% for R. patientia and 92% for R. obtusifolius.

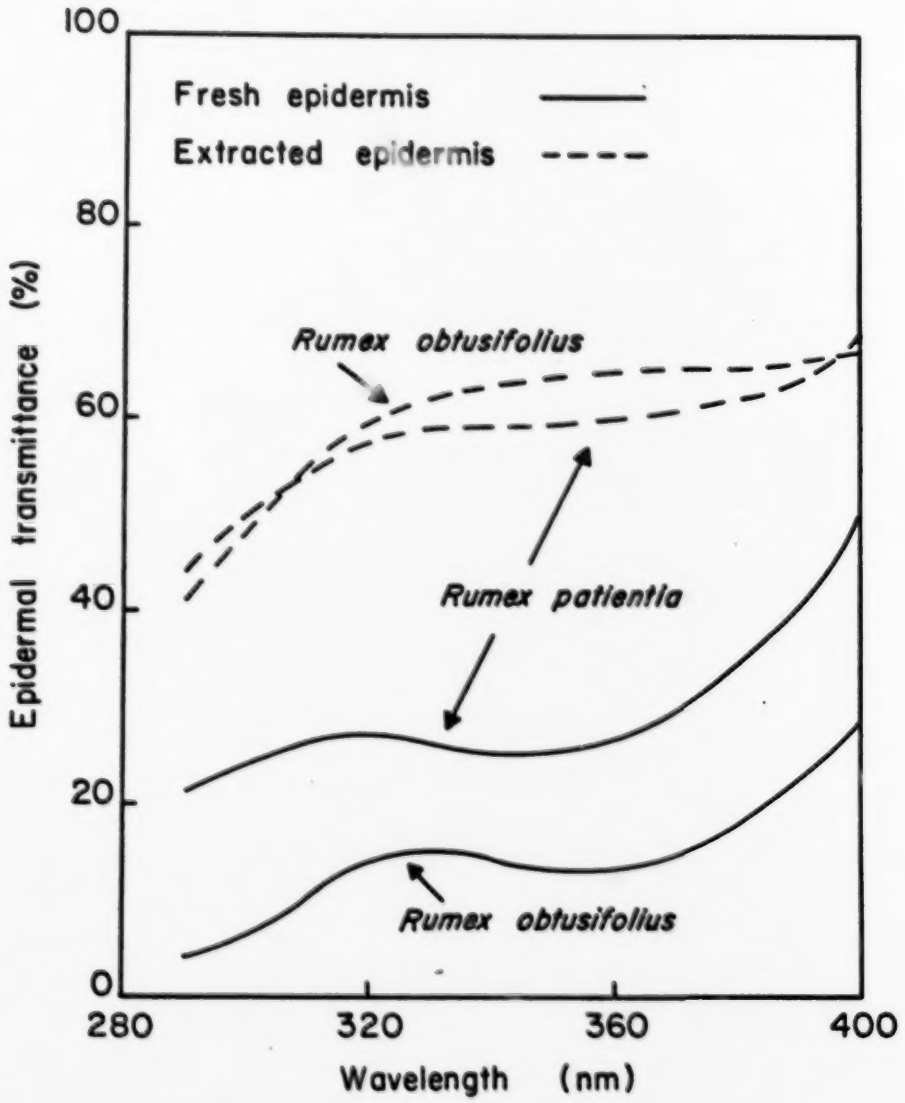

Fig. 3. Epidermal UV transmittance of fresh tissue and of tissue from which UV-absorbing pigments were extracted. The species were collected from the field. Mean transmittance of 5 and 10 samples per spectrum are shown for Rumex obtusifolius and R. patientia, respectively.

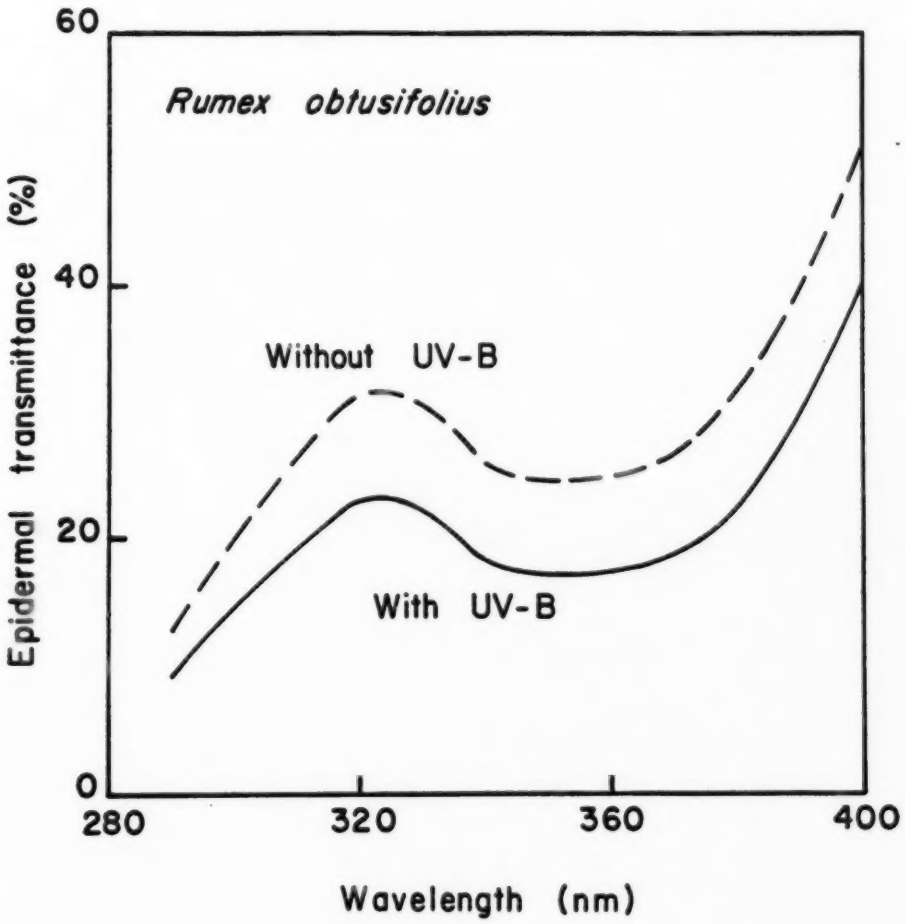

Fig. 4. Epidermal UV transmittance of Rumex obtusifolius leaves with or without solar UV-B irradiation. The plants were exposed to solar UV-B radiation at a mean biologically effective dose rate of 1151 J m^{-2} d^{-1} for eight days. These spectra represent the mean of nine samples per treatment. Epidermal transmittance of UV-B-irradiated leaves was significantly lower ($p \leq 0.05$) than that of nonirradiated leaves.

Table 1. Extract absorbance of whole-leaf tissue for two Rumex species exposed to solar UV-B radiation. The mean of three to five samples per treatment for each species is presented as absorbance per mg dry weight. Significant increases ($p \leq 0.05$) in absorbance are indicated by an asterisk.

Species	Days of solar UV-B irradiation	Mean UV-B daily dose (Biologically effective J m^{-2} d^{-1})	Absorbance (at 305 nm)	
			No UV-B	With UV-B
R. patientia	5	1125	0.27	0.30*
R. obtusifolius	8	1151	0.26	0.50*

DISCUSSION

Reflectance of UV-B radiation from the leaf surface appears to be of limited value for preventing the penetration of this radiation into the leaf. The relatively low and nonwavelength-selective reflectance of 4%, found for the two Rumex species in this study, is typical for glabrous leaves (Gausman et al. 1975; Robberecht et al. 1980). Leaf surfaces covered with certain types of heavy pubescence or cuticular waxes can reflect more than 40% of the UV-B radiation incident on the leaf surface. Notable examples of high UV-B reflectance are Dudleya brittonia (Mulroy 1979) and Argyroxiphium sandwicense (Robberecht et al. 1980). Reflectance in these rather uncommon cases is relatively nonselective for wavelength, and is generally high in visible as well as UV wavebands. No significant increase or decrease in reflectance has been found in response to UV-B irradiation (Gausman and Escobar 1982).

The epidermis is a highly effective filter for UV-B radiation for both Rumex species examined in this study. Similar levels of UV attenuation have been observed for other species (Lautenschlager-Fleury 1955; McCree and Keener 1974; Gausman et al. 1975), though UV attenuation of up to 99% has been reported (Robberecht et al. 1980). For Rumex patientia and R. obtusifolius, as well as for Oenothera stricta (Robberecht and Caldwell 1983), nearly all of the UV-B radiation that penetrates through the epidermis is absorbed in the mesophyll layer. Thus, whole leaves appear to be opaque to UV-B radiation.

Increased epidermal attenuation in Rumex obtusifolius after solar UV-B irradiation indicates a degree of plasticity in epidermal optical properties. Such plasticity has been documented in other species (Lautenschlager-Fleury 1955; Robberecht and Caldwell 1983). The increased epidermal UV-attenuation capacity found for Rumex obtusifolius was most likely due to an increase in UV-absorbing pigments. Flavonoid and related phenolic compounds can be induced by UV irradiation (Wellmann 1974, 1975). Further, flavonoid extracts from Rumex leaves showed significant increases in absorbance after UV-B irradiation. This has also been reported for other species (Sisson 1981; Robberecht and Caldwell 1983). The substantial difference in fresh epidermal transmittance between the two Rumex species

is most likely related to the concentration of UV-absorbing compounds in the tissue, since after extraction of these compounds the spectra were similar (see Fig. 3).

The results of this study, along with the investigations of Caldwell (1968), Wellmann (1974, 1975), Sisson (1981), and Robberecht and Caldwell (1983), strongly suggest that the epidermis has a significant protective role against UV-B induced damage to sensitive physiological targets in the mesophyll. Furthermore, flavonoid and related phenolic compounds are most likely the major sources of UV-B absorption in the epidermis, and to some extent in the mesophyll. Since these compounds generally do not absorb visible radiation (Mabry et al. 1970: McClure 1976), their presence in leaf tissue provides a viable protective mechanism against potential damage from UV-B radiation. A mechanism involving the induction of flavonoids in response to UV-B irradiation results in a leaf epidermis that is an effective filter for UV-B radiation, has the plasticity for increased attenuation, and is highly selective for wavelength. In an intensified UV-B radiation environment expected to occur with stratospheric ozone depletion, such a protective mechanism could have particular adaptive significance. Predictions of plant sensitivity in the intensified UV-B environment, however, are difficult because the UV-attenuation capacity and prevalence of the protective mechanism in species of different plant communities is not known (Caldwell et al. 1983).

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REFERENCES

Biggs RH, Sisson WB, Caldwell MM (1975) Response of higher terrestrial plants to elevated UV-B irradiance. In: Nachtwey DS, Caldwell MM, Biggs RH (eds) Impacts of climatic change on the biosphere, part I, ultraviolet radiation effects, monograph 5. Climatic Impact Assessment Program, US Dept Transportation Report No DOT-TST-75-55, NTIS, Springfield, Virginia, p (4)34

Caldwell MM (1968) Solar ultraviolet radiation as an ecological factor for alpine plants. Ecol Monogr 38:243-268

Caldwell MM (1971) Solar UV irradiation and the growth and development of higher plants. In: Giese AC (ed) Photophysiology, vol 6. Academic Press, New York, p 131

Caldwell MM, Robberecht R, Flint SD (1983) Internal filters: prospects for UV-acclimation in higher plants. Physiol Plant 58:445-450

Gausman HW, Escobar DE (1982) Reflectance measurement of artificially induced ultraviolet radiation stress on cotton leaves. Rem Sens Environ 12:485-490

Gausman HW, Rodriquez RR, Escobar DE (1975) Ultraviolet radiation reflectance, transmittance, and absorptance by plant epidermises. Agron J 67:719-724

Lautenschlager-Fleury D (1955) Über die Ultraviolettdurchlässigkeit von Blattepidermen. Ber Schweiz Bot Ges 65:343-386

Lee DW, Lowry JB (1980) Solar ultraviolet on tropical mountains: can it affect plant speciation? Am Nat 115:880-883

Mabry TJ, Markham KR, Thomas MB (1970) The systemic identification of flavonoids. Springer, Berlin Heidelberg New York Tokyo

McClure JW (1976) Progress toward a biological rationale for the flavonoids. Nova Acta Leopoldina, Suppl 7:463-496

McCree KJ, Keener ME (1974) Effect of atmospheric turbidity on the photosynthetic rates of leaves. Agric Met 13:349-357

Mulroy TW (1979) Spectral properties of heavily glaucous and non-glaucous leaves of a succulent rosette-plant. Oecologia (Berl) 38:349-357

National Academy of Sciences (NAS) (1982) Causes and effects of stratospheric ozone reduction: an update. Washington DC, p 145, ISBN 0-309-03248-2

Ribereau-Gayon P (1972) Plant phenolics. Hafner, New York, ISBN 0-05-0025-120

Robberecht R, Caldwell MM (1978) Leaf epidermal transmittance of ultraviolet violet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. Oecologia (Berl) 32:277-287

Robberecht R, Caldwell MM (1983) Protective mechanisms and acclimation to solar ultraviolet-B radiation in Oenothera stricta. Plant Cell Environ 6:477-485

Robberecht R, Caldwell MM, Billings WD (1980) Leaf ultraviolet optical properties along a latitudinal gradient in the arctic-alpine life zone. Ecology 61:612-619

Sisson WB (1981) Photosynthesis, growth, and ultraviolet irradiance absorbance of Cucurbita pepo L. leaves exposed to ultraviolet-B radiation (280-315 nm). Plant Physiol 67:120-124

Sisson WB, Caldwell MM (1976) Photosynthesis, dark respiration, and growth of Rumex patienta L. exposed to ultraviolet irradiance (288 to 315 nanometers) simulating a reduced atmospheric ozone column. Plant Physiol 58:563-568

Teramura AH (1983) Effects of ultraviolet-B radiation on the growth and yield of crop plants. Physiol Plant 58:415-427

Van TK, Garrard LA (1975) Effect of UV-B radiation on net photosynthesis of some C_3 and C_4 crop plants. Soil Crop Sci Soc Florida Proc 35:1-3

Wellmann E (1974) Regulation der Flavonoidbiosynthese durch ultraviolettes Licht und Phytochrom in Zellkulturen und Keimlingen von Petersilie (Petroselinum hortense Hoffm.). Ber Dtsch Bot Ges 87:267-273

Wellmann E (1975) UV dose-dependent induction of enzymes related to flavonoid biosynthesis in cell suspension cultures of parsley. FEBS Lett 51:105-107